Pharmacology Biochemistry & Behavior, Vol. 18, pp. 777–784, 1983.

Addictive Agents and Intracranial Stimulation (ICS): Novel antagonists and Agonists of Morphine and Pressing for ICS

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Received 31 October 1980

BERMUDEZ-RATTONI, F., S. CRUZ-MORALES AND L. D. REID. *Addictive agents and intracranial stimulation (ICS): Novel antagonists and agonists of morphine and pressing for ICS.* PHARMACOL BIOCHEM BEHAV **18**(5) 777–784, 1983.—Rats fixed with chronically indwelling bipolar electrodes pressed for intracranial stimulation (ICS) of the lateral hypothalamus during daily sessions. The effects of two antagonists of morphine (Win 44,441 and naloxone) were then assessed. Naloxone (10 mg/kg) produced its characteristic reduction in pressing. Win 44,441 produced a reliable increase in pressing at doses as small as 1 mg/kg. Large doses of morphine (10 mg/kg) produced its characteristic effects: depression in pressing when given 1 hr before the test session and facilitation when given 3 hr before the test session. Win 44,441 antagonized morphine's depressive effects. Other compounds (Win 44,156, Win 42,156), having similar structure to Win 44,441 but having agonist and mixed agonist-antagonist activity with respect to analgesia, also facilitated pressing for ICS. All three compounds' effects on pressing for ICS were antagonized by naloxone. It is inferred that opioids' facilitatory effects on pressing for ICS are separable from opioids' other capabilities such as production of analgesia.

Lateral hypothalamus N-Methylbenzomorphans Rewarding brain stimulation Naloxone Endorphins Opioid receptors

STUDIES of opioids and mechanisms of reinforcement are interesting because of their potential relevance to theories of drug addiction. Studies of opioids and motivated behavior take on new relevance, however, in light of recent discoveries indicating that there is an extensive and intricate endogenous morphine-like system (for recent chronicles of progress, see [26] and *Life Sci.* Suppl. **31**: 1982). This endogenous morphine-like system apparently has special relevance to motivation and emotion [3, 8, 11, 19, 23].

The idea that addicting opiates, such as morphine and heroin, act on neural systems that are integral to reinforcement from direct electrical stimulation of brain has received considerable experimental support [8]. Morphine and heroin, in doses that might be self-administered, increase pressing for intracranial stimulation (ICS) [1, 5, 22] and lower threshold for intracranial reinforcement [7,13]. A wide variety of other potentially addicting opioids produce similar effects. At the time when morphine produces its facilitative-effects on pressing for ICS, independent behavioral tests indicate that the morphine is producing a positive affective state [24].

Since naloxone is the prototypic antagonist of morphine-like effects, it would be expected that naloxone would counter the effects of morphine on pressing for ICS. Naloxone does, indeed, reverse the effects of morphine [4,20]. Also, if the endogenous opioid peptide system is part of the system activated by reinforcing ICS, naloxone should modify pressing for ICS in opioid-naive subjects, since naloxone also antagonizes the effects of the endogenous opioids. Naloxone does, indeed, reduce pressing for ICS in otherwise opioid-free subjects [2,25].

Naloxone, in doses as small as 1 mg/kg, reliably decreases pressing for ICS in opioid-free subjects [2]. The extent of that reduction, however, is relatively small and pressing is hardly ever abolished by administration of naloxone. Because naloxone only attenuates pressing for ICS, it was

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concluded that endogenous elements modulated positive intracranial reinforcement, but were not critical [25]. Such a conclusion is consistent with the general observations that naloxone, in opioid-free subjects, has few effects [12]. Naloxone only has dramatic effects when subjects are tested in special circumstances [23].

The experiments to be reported here involve tests for pressing for ICS in rats with a novel morphine antagonist, Win 44,441. The experiments were begun with the rationale that further support for the idea of involvement of endogenous elements in the system of intracranial reinforcement would be strengthened by findings that antagonists other than naloxone would decrease pressing for ICS in otherwise opioid-free subjects [10]. To our surprise, opiate antagonists have far from uniform effects on pressing for ICS. This led us to rethink our original rationale and lead us to the conclusion that there were relatively specific endogenous components modifying the intracranial reward system.

Win 44,441 is an N-methylbenzomorphan narcotic antagonist. It shows no agonist activity in the Guinea-pig ileum or mouse vas deferens assays. It is inactive, i.e., produces no signs of analgesia, in the standard tail-flick or intracarotid bradykinin tests. Win 44,441 antagonizes the effects of analgesic opiates [18]. Win 44,441 has somewhat decreased antagonistic potency in the rat vas deferens compared to the Guinea-pig ileum; but, in doses used in these studies, it is anticipated that such differential sensitivity may not be a factor [18,27]. From initial assays, Win 44,441 appears to be a pure antagonist having properties similar to naloxone.

In addition to tests with Win 44,441, other tests with compounds of the same type were conducted. There were tests of the effects of Win 44,156, an agonist, and of Win 42,156, a mixed agonist and antagonist. Both of these compounds also facilitated pressing for ICS. Consequently, all three types of compounds of this class of opioids (antagonist, agonist, mixed agonist-antagonist with respect to analgesia) facilitated pressing for ICS. Analgesic properties of the compounds, therefore, do not covary with their effects on pressing for ICS.

GENERAL METHOD

Subjects

Subjects were adult, male rats (Taconic Farms, Sprague-Dawley derived) weighing 250 to 450 g at the beginning of the procedures. Each was fixed, using standard procedures, with a chronically indwelling bipolar electrode (Plastic Products) for ICS of the lateral hypothalamus. Each strand of the bipolar electrode was insulated except at the cross section of the stimulating tip. The two strands (0.25 mm diameter/strand) were separated from one another only by the width of the insulation.

The coordinates for the stimulating tips of the electrodes were 3.3 mm posterior to bregma, 1.5 mm lateral to the midline, and 8.2 mm below the skull's surface. The rat was positioned in the stereotaxic so that bregma and lambda were in the same horizontal plane with the electrode shaft perpendicular to that horizontal plane. Subsequent histological procedures verified that the electrode tips were all within the area of the lateral hypothalamus. The most frequent site of ICS was the medial forebrain bundle with some stimulation sites involving the zona incerta. All electrode tips were lateral to the fornix, medial to the internal capsule and ventral to the medial lemniscus. The anterior-posterior extent of electrode sites was similar to boundaries of the ventromedial n.

Throughout the procedures, rats were housed individually with food and water always available. The colony room was maintained at 24°C on a reverse light-dim light cycle (LD 12:12). The dim cycle began at 1000 hr and subjects were tested from 1230 to 1530 hr in Experiments 1, 2, and 3 and from 0930 to 1200 hr in the other experiments.

Apparatus

The subjects' rates of pressing for ICS were tested in six nearly identical experimental chambers. Each chamber was a clear plastic box (30×24×5 cm) fitted with a lever extending from one wall, 7.5 cm above the floor. Each depression of the lever resulted in the delivery of ICS. Each train of ICS was 0.3 sec of 60 Hz sine waves. Current intensities were selected specifically for each subject, but were always lower than 50 microA. Press rates were recorded automatically for each 5-min period across a 1-hr testing session.

Procedure

The surgery of implanting the electrodes was done under sodium pentobarbital anethesia (45 mg/kg) with atropine sulfate as an adjunct. After at least 5 days for recovery from surgery, each subject was trained to press for ICS. During initial daily training sessions, intensity of ICS was varied and an intensity selected for each subject. The selected intensity maintained moderate press rates (about 50/min) without side effects such as seizures or gross forced movements. The intensities ranged from 16 to 40 μA. Once an intensity was selected for a subject, it remained constant throughout the remainder of all testing.

After selecting an intensity of ICS, each rat was tested daily in 45-min or 1-hr sessions, for at least 2 weeks, so that press rates stabilized. After pressing stabilized (mean press rates varying not more than 10% across 3 days), injections were given before a testing session but all other features remained the same.

Prior to a test of a specific drug, subjects were tested while under the influence of an injection of physiological saline, or sterile water, the carrier of the drugs. Sessions with these placebo injections also occurred after a test with a drug. Because of the possibility that the Win compounds have lasting effects, only predrug placebo scores were used as standards for comparison. Subjects' mean press rates did not differ statistically on days under predrug placebo when grouped as they eventually received a drug.

Press rates were measured each 5-min across a daily session. Initial analyses, using analyses of variance for repeated measures, indicated press rates reliably, but slightly, declined across a daily session, but in no instance was there a reliable interaction between the factor of measures across a session and the factor associated with drug-effects. Consequently, for simplicity, all analyses were eventually done with mean presses/5 min. Student's t-tests, for paired comparisons when appropriate (e.g., mean pressing under predrug placebo to mean pressing under drug), were used to assess statistical significance.

At the conclusion of the behavioral testing, the animals were sacrificed with overdoses of penobarbital, and perfused with saline and Formalin (10%). The brains were removed and subsequently sectioned (90 μ). The sections were then treated as photographic negatives and enlarged photographs taken to aid in determination of site of stimulation.

EXPERIMENT 1

This was the first test with the compounds Win 44,441-2 and -3 in the self-stimulation procedure.

METHOD

Ten subjects were tested. After predrug placebo sessions, one-half of the subjects were tested under the influence of Win 44,441-3 (the levo isomer) while the others were tested under Win 44,441-2 (the dextro isomer). The injections were given 1 hr before the test-session in doses of 10 mg/kg, subcutaneously (SC). The carrier was sterile water and all injections were 1 ml/kg.

After the post drug placebo sessions, the rats were again tested with the compounds except those that had previously been give dextro were given levo. Consequently, each rat was tested under each isomer and tests with each isomer can be compared to tests with placebo.

RESULTS AND DISCUSSION

A summary of the results are depicted in the left side of Fig. 1. The levo isomer of Win 44,441 increased pressing for ICS compared to pressing of the predrug placebo day, $t(9)=3.38$, $p<0.01$. Win 44,441-2 (dextro) however, did not significantly affect pressing rates, $t(9)=0.81$.

Since Win 44,441-3 (levo) is an antagonist, we expected an effect similar to that produced by naloxone, i.e., a decrease in pressing. The Win compound, however, increased press rates for ICS while the inactive isomer had little or no effect.

Because of the results of the previous experiment were not expected, the basic procedures for testing for the effects of the Win compound were repeated using five experimentally naive rats. As expected from results of Experiment 1, the Win compound (44,441-3, levo) reliably increased pressing. When scores of the test with Win were compared with the scores from previous placebo, the resulting $t(4)=3.17$, $p<0.02$ (the mean under placebo=280.28/5 min; the mean under levo-Win 414.96/5 min). There were no reliable differences between scores following the inactive Win compound compared with the placebo day (mean of placebo-day=291.75/5 min; mean under dextro-Win=271.22/5 min). These data verify the initial observation showing that the "antagonist," Win 44,441-3, facilitates pressing for lateral hypothalamic ICS.

EXPERIMENT 2

Since the active isomer of the Win compound produced the surprising result of facilitation in pressing for ICS, and since results with naloxone show considerable individual differences, it is important to test the effects of naloxone in the same subjects that the Win compound was tested. Naloxone should produce small but reliable decreases in pressing [25].

METHOD

Five days after the last injection of the Win compound, the procedures testing for the effects of naloxone were begun using nine of the subjects of Experiment 1 (one subject's electrode became loose). The subjects continued to press daily for their selected ICS, as usual, in 1-hr sessions while injections were given before daily testing sessions. Following predrug placebo tests, four of the subjects were given naloxone hydrochloride (10 mg/kg, SC), 1 hr before the session, while the other five received placebo injections. Subsequently, the naloxone was given to the group receiving placebo and the group receiving naloxone received placebo.

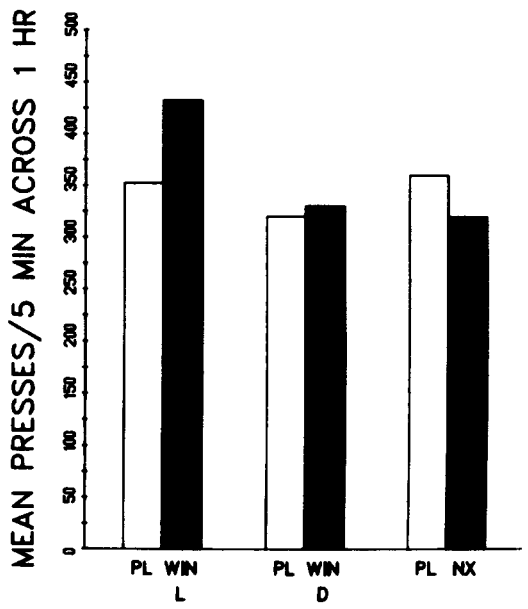

FIG. 1. Mean presses for lateral hypothalamic ICS. PL=presses under placebo injections; WIN/L=presses with WIN 44,441 levo; WIN/D=presses with WIN 44,441 dextro; NX=presses with naloxone.

RESULTS AND DISCUSSION

Naloxone reduced pressing for ICS (Fig. 1, right). The reduction from predrug placebo was 10%, $t(8)=2.12$, $p=0.03$, one tailed. Also, when the press rates with naloxone were compared to those with Win 44,441-3 (levo) in the same subjects, we found statistically significant differences between rate of pressing, $t(8)=2.64$, $p<0.05$. It is difficult to conclude that the two putative antagonists are acting in the same way on pressing for ICS even though they both antagonize opioid analgesia.

EXPERIMENT 3

The effects of morphine on pressing for ICS are known. Large doses (e.g., 10 mg/kg) produce a depression in pressing when the tests occur 1 hr after injections but produce facilitation in pressing when tests occur about 3 hr after injections [1,6]. Both the initial depression and the subsequent facilitation are reversed by naloxone [4,20]. The question addressed in this experiment is whether or not the Win compound will also antagonize the effects of morphine on pressing for ICS.

METHOD

Five days after the last injection of naloxone, seven rats of the previous experiment began the procedures of this experiment. As usual, they began the procedures with a series of tests following placebo injections. Then, 1 hr before a session, all subjects received a 10 mg/kg injection of morphine sulfate, a procedure designed to produce a marked depression of pressing. The next day, subjects again received the same morphine injection but also received a 10

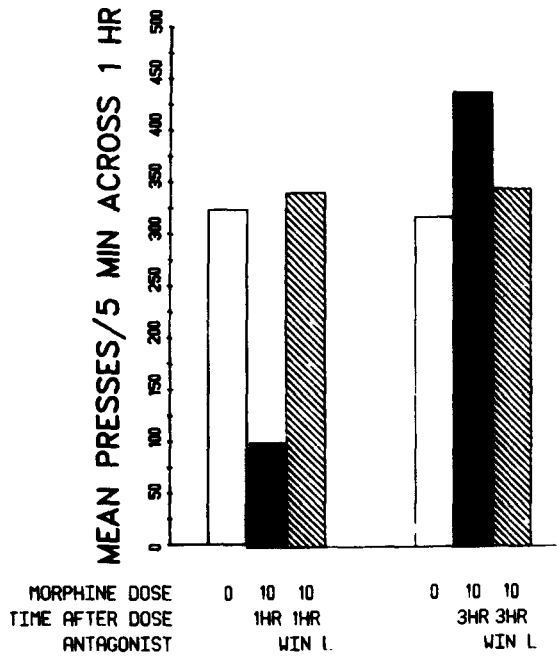

FIG. 2. Mean presses for ICS showing effects of morphine, 10 mg/kg, at two times after injections with and without additional injections of WIN 44,441. Pressing with the WIN compound is depicted by the right bar of the three bars.

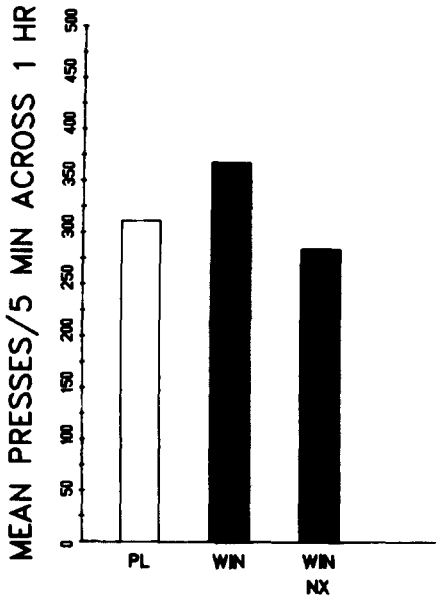

FIG. 3. Mean presses for ICS when subjects were under the influence of placebo (PL), WIN 44,441 (WIN) and both WIN and naloxone (NX).

mg/kg dose of Win 44,441-3 (levo) immediately after the morphine injection.

After another series of placebo injections (2 daily sessions), morphine (10 mg/kg, SC) was given again, but this time 3 hr before the testing. This procedure was designed to produce facilitation of pressing. The next day, morphine was given again 3 hr before the session and Win 44,441-3 was given 1 hr before.

RESULTS AND DISCUSSION

As expected, rats receiving morphine 1 hr before testing decreased pressing compared to pressing under placebo, $t(6)=2.91$, $p<0.01$ (Fig. 2, left). The next day when the Win compound was also administered, there was no reliable difference between pressing under the effects of the two drugs compared to pressing under placebo $t(6)=1.69$, $p>0.10$. There was a statistically significant difference between scores following morphine alone and morphine plus Win compound, $t(6)=2.65$, $p<0.03$. When morphine was administered 3 hr before testing, it produced a reliable increase in pressing, $t(6)=5.30$, $p<0.001$ (Fig. 2, right). Although there was a slight increment in pressing, when the Win compound plus morphine were administered, there were no reliable differences compared with the placebo day, $t(6)=1.70$, $p>0.10$. The comparison between scores under morphine and under morphine plus Win compound yielded a $t(6)=6.07$, $p<0.001$.

Morphine produced its characteristic effects on pressing for ICS, depression in pressing shortly after a large dose and subsequently a facilitation in pressing. Win 44,441 completely antagonized the depressive effects of morphine and attenuated morphine's facilitation. There is the question of why morphine plus Win 44,441 did not, in combination, produce a statistically significant increment in pressing. There are a number of potential procedural problems including the possibility of a mild nonspecific toxicity dampening the facilitation and the small number of tests and subjects. Only further study of dose-response relationships of each drug and drugs in combination will allow clarification of the question concerning the lack of reliable facilitation with the two drugs of combination.

Despite some potential procedural problems, some definite conclusions can be drawn. Win 44,441 antagonizes morphine's depressive effects on pressing for ICS. Yet, Win 44,441 will, by itself, facilitate pressing. From these findings, it is difficult to conclude that the depressive effects of opioids are necessary for the facilitation of pressing to emerge. We infer, therefore, that morphine's facilitation is not a rebound from its depressive effects but rather due to the activation of a subclass of receptors that, in turn, either directly or indirectly, affect pressing for ICS.

EXPERIMENT 4

In this experiment, we tested whether naloxone would antagonize the facilitative effects of Win 44,441-3. If naloxone antagonizes the facilitation effects of Win 44,441, then there will be further evidence that the effects seen in these experiments involve opioid receptors, or, at least, naloxone-sensitive receptors. Also, various doses of Win 44,441-3 were tested.

METHOD

Nine, experimentally naive, male rats weighing 390 to 450 g were fixed with electrodes, were trained and tested as the subjects of Experiment 1. They were given training in the bar press situation, until response rates stabilized, and then continued their daily pressing for a fixed intensity of ICS while they received drugs before a daily test.

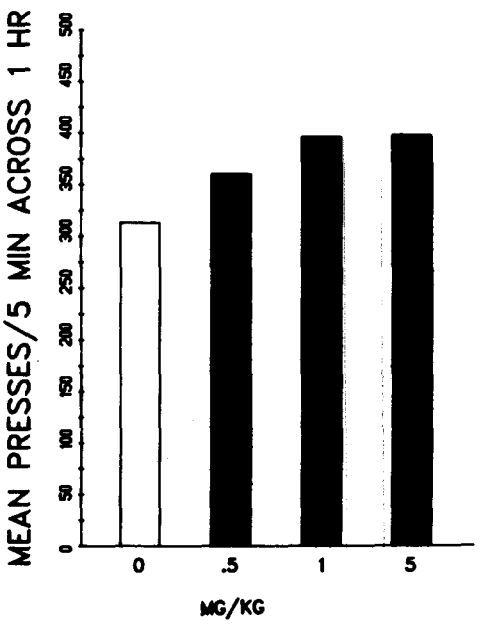

FIG. 4. Mean presses for ICS when subjects were under the influence of various doses of WIN 44,441.

Five subjects were given Win 44,441-3 (10 mg/kg, SC) and then, before a subsequent session, Win 44,441-3 (10 mg/kg, SC) plus naloxone (10 mg/kg, SC). Four were given the same drugs but in the reverse order. When Win 44,441 was given, it was 1 hr before the test. When naloxone was given, it was given 15 min before the test. At least 2 days of testing under physiological saline (placebo) proceeded each day of testing under opioids and 4 days of testing intervened between tests under opioids. Inspection of the data indicated that order of testing was an insignificant source of variance. Also, there were no reliable differences among scores on days of placebo. Consequently, the data were reduced by averaging across all placebo scores to get a single index of pressing under placebo, and order of testing with opioids was ignored, yielding a 1 by 3 analysis of variance design having repeated measures and a factor for subjects and a factor for each drug treatment (placebo, Win 44,441, and Win 44,441 plus naloxone).

The same subjects continued their daily sessions with ICS as various doses of Win 44,441 were given. The test sessions, however, were 45 min rather than 1 hr. Between each test with a particular dose, 4 days of tests were programmed under the influence of physiological saline. The dose of Win that were given were 5, 1, and 0.5 mg/kg. Scores of predrug placebo tests (2 days before drug) did not differ significantly across this series of tests. Consequently, the mean of predrug placebos were taken as the best index of the effects of 0 mg/kg.

RESULTS AND DISCUSSION

The results are summarized in Fig. 3. An ANOVA of the data used to derive the figure yielded an $F(2,16)=15.9$, $p<0.001$, for the factor of drugs. Again the Win compound produced an increase in pressing, about 19%, compared to placebo, $t(8)=3.96$, $p<0.025$. When Win 44,441 and naloxone were given, the subjects pressed as would be expected had they been given naloxone alone, i.e., their press rates were decreased, about 8%, compared to placebo, $t(8)=2.31$, $p<0.05$.

Mean press rates with 0, 0.5, 1, and 5 mg/kg doses of Win 44,441 are depicted in Fig. 4. The dose of 1 and 5 mg/kg produced 26% and 27% increases in pressing, respectively, compared to placebo scores, $t(8)=2.27$, $p<0.05$, and $t(8)=5.14$, $p<0.005$, respectively. The dose of 0.5 mg/kg produced an increase of about 15%, however, that increase was not sufficiently uniform to produce a statistically significant difference from placebo, $t(8)=1.3$, $p<0.25$.

It is clear, Win 44,441 produces increases in pressing for hypothalamic ICS. The increase was demonstrated in three different sets of subjects and in four different procedures. The increase is related to the stereospecificity of the drug and it is reversible by naloxone. The increase occurs with relatively small doses. Furthermore, the Win compound was capable of reversing the suppressant effects of large doses of morphine. In brief, Win 44,441 is an antagonist of many of morphine's effects, but facilitates pressing for ICS.

EXPERIMENT 5

This experiment is concerned with the effects of Win 44,156 on pressing for ICS of the lateral hypothalamus. Win 44,156 is another N-methylbenzomorphan. It is strongly analgesic using the rat tail-flick, intracarotid bradykinin and Ach-writhing tests. It is active in the Guinea pig ileum and rat vas deferens bioassays and is antagonized by naloxone. Prior to these tests, there were informal observations of Win 44,156's effects. Doses of 2 and 5 mg/kg, SC, produced clear signs of analgesia using the tail flick measure. A dose of 10 mg/kg, SC, produced marked catatonia lasting about 30 min and beginning about 15 min after injections. Given these observations, it was decided to start testing with doses less than 0.5 mg/kg in the self-stimulation test, since it would be expected that only doses smaller than doses producing catatonia would facilitate pressing for ICS. Also, the effects of Win 44,441 and naloxone were tested for their effects following administration of Win 44,156.

METHOD

Eight subjects that were used in Experiment 4 continued daily pressing for ICS and served as the subjects of these tests. Six days after the last test with Win 44,441 and naloxone, the subjects began the tests of these procedures.

As is usual, all features of a daily session with ICS remained the same except that injections were given before a session, i.e., subjects were tested at the same time of day (0900 to 1200 hr), in the same experimental chambers, and with the same ICS (60 Hz sine waves of 0.3 sec duration with intensity ranging from 18 to 45 microampers but constant for a given subject). On day before a test using Win 44,156, all rats were given an injection of placebo, physiological saline. All injections were 1 ml/kg, SC, 0.5 hr before the beginning a 45 min-session with ICS.

On the day following the first placebo injections, one half of the subjects received 0.5 mg/kg of Win 44,156, and the other half received 0.25 mg/kg of Win 44,156. Four days later, the procedure were repeated except the subjects receiving 0.5 mg/kg received 0.25 mg/kg and those receiving 0.25 mg/kg got 0.5 mg/kg. Since the results indicated that these two doses produced facilitation of pressing, further tests were programmed using a smaller dose.

Four days after the tests with 0.25 and 0.5 mg/kg during which all subjects received placebos, all subjects were given

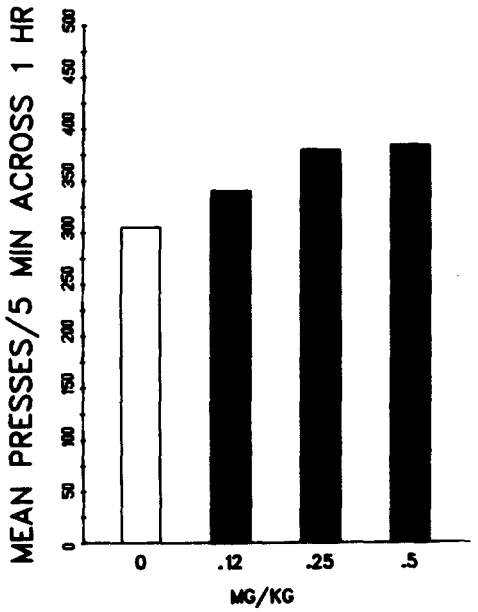

FIG. 5. Mean presses for ICS when subjects were under the influence of various doses of WIN 44,156.

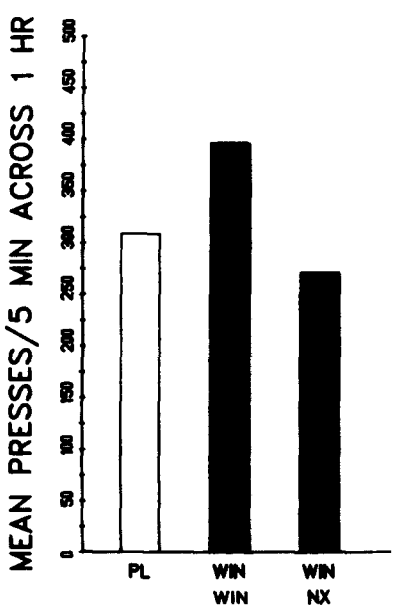

FIG. 6. Mean presses for ICS when subjects were under the influence of (PL) placebo; (WIN/WIN) WIN 44,156 and WIN 44,441; and (WIN/NX) WIN 44,156 and naloxone.

a 0.125 mg/kg dose of 44,156, SC. The entire sequence was followed by 4 more daily sessions with placebo.

These subjects' press rates remained stable across the procedure when they recevied placebo before each treatment. Consequently, mean predrug placebo scores were used (mean pressing for 5 min across 45-min sessions and across all predrug placebo sessions) to assess the effects of 0 mg/kg. Also an ANOVA with repeated measures indicated that the sequence of testing was an insignificant source of variance.

After the initial tests of Win 44,156, rats continued their daily sessions with ICS as before. On a test day, all subjects were given Win 44,156 (0.5 mg/kg, 30 min before test session) and then one half were given naloxone (4 mg/kg, 15 min before) while the other half received Win 44,441 (1 mg/kg, 60 min before). All subjects received placebo the day before. All injections were 1 ml/kg, SC. Four days later there was another test, but this time those subjects receiving naloxone received Win 44,441 and vice versa.

To get a score for placebo effects, all predrug placebo scores were averaged since they did not vary reliably across tests. Consequently, these second set of tests yielded an index of the effects of (a) placebos, (b) Win 44,156 plus Win 44,441, and (c) Win 44,156 plus naloxone, in terms of mean presses/5 min across 8 subjects. From initial tests, there is an index of the effects of Win 44,156 by itself and from previous research we can estimate the effects of naloxone by itself.

RESULTS AND DISCUSSION

Win 44,156, at the higher doses, clearly facilitated pressing compared to the predrug placebo scores (Fig. 5). An ANOVA of the scores used to derive Fig. 5 yielded an $F(3,21)=3.9$, $p<0.023$ for the factor associated with doses of drug. Student's t-tests comparing responses under Win 44,156 and responding under placebo yielded $t(7)=2.59$, $p<0.031$; $t(7)=4.26$, $p<0.022$, and, $t(7)=2.05$, $p<0.06$, for 0.5, 0.25, and 0.125 mg/kg, respectively.

As can be seen (Fig. 6), Win 44,156 plus naloxone leads to decreased pressing compared to mean predrug placebo scores. Win 44,156 plus Win 44,441 leads to increased pressing. An ANOVA, having repeated measures, of the scores used to derive Fig. 6 yielded an $F(2,14)=18.9$, $p<0.001$. By t-tests, all possible pairs of means are reliably different from one another.

EXPERIMENT 6

As mentioned, it is of interest to test drugs of the N-methylbenzomporphan class because of their different profile of effects in many tests but their apparent similarity of effects on pressing for ICS. Win 42,156 is another N-methylbenzomorphan, presenting potent phenazocine antagonist activity, but presenting strong agonist properties in acetylcholine and bradykinin test and is inactive with the tail flick test [18]. Win 42,156, therefore, would be characterized as a mixed agonist-antagonist. These were the first tests of Win 42,156 in the self-stimulation test.

METHOD

The format of the following procedures is very similar to that of previous tests. Nine experimentally naive subjects were used. They were fixed with electrodes and trained to press for ICS. After press rates stabilized, various doses of Win 42,156 were given: 0.0, 0.125, 0.25, 0.5 and 1.0 mg/kg, SC. As before, a predrug placebo was given a day before each administration of the Win compound.

The first two doses were 0.5 and 1.0 mg/kg and the second two were 0.125 and 0.25. Five of the subjects received one of the two doses on a test day while the other four were given the other dose. After 4 days with placebo conditions, the test was repeated, the dose given first to the five was given second to the other four. Dosing continued until all subjects received every dose. Four days without drug intervened between each administration.

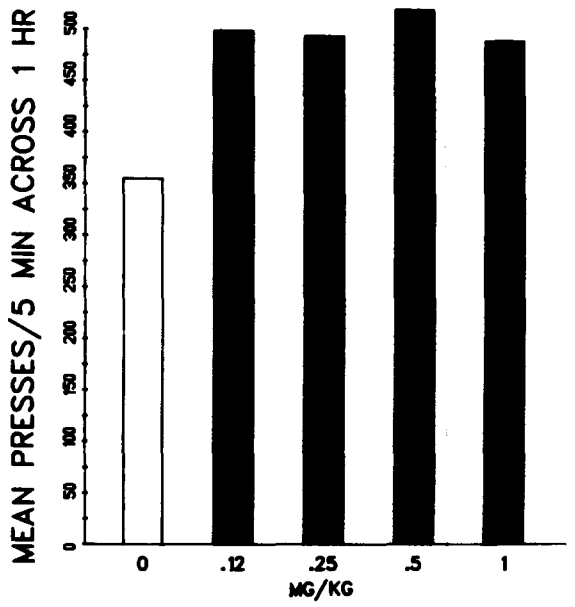

FIG. 7. Mean presses for ICS when subjects were under the influence of various doses of WIN 42,156.

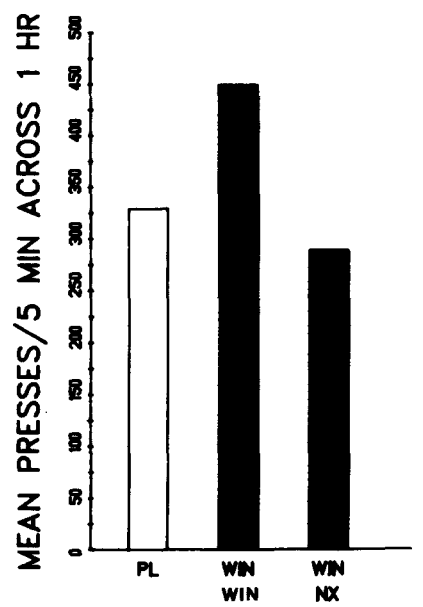

FIG. 8. Mean presses for ICS when subjects were under the influence of (PL) placebo; (WIN/WIN) WIN 42,156 and WIN 44,441; and (WIN/NX) WIN 42,156 and naloxone.

As with the previously tested drug, the effects of Win 42,156 were tested with naloxone and Win 44,441. Eight rats of the previous test were used. The dose of Win 42,156 that was used was 0.5 mg/kg, SC, because that dose clearly increased pressing. The dose of naloxone and Win 44,441 were the same doses as in the previous experiment, i.e., 4 and 1 mg/kg of naloxone and Win 44,441, respectively.

RESULTS AND DISCUSSION

The results were summarized in Figs. 7 and 8. An ANOVA of the data used to derive Fig. 7 yielded an $F(4,32)=7.31, p<0.001$. All scores of the Win compound are reliably different from scores of placebo, $ps<0.03$, and not different from each other. Clearly, Win 42,156 reliably increases press rates for ICS.

It can be seen (Fig. 8), that naloxone reversed the effect of Win 42,156; but with Win 44,441, the response rates were only slightly reduced compared to response rates produced by Win 42,156 alone. An ANOVA of the data of Fig. 8 yielded an $F(2,14)=6.82, p<0.009$. Win 42,156 plus naloxone leads to decreased pressing compared to mean predrug placebo scores, $t(7)=3.68, p<0.01$. Win 42,156 plus Win 44,441 leads to increased pressing rats, $t(7)=2.32, p<0.04$.

GENERAL DISCUSSION

Rate of baseline pressing will affect measures of drug effects on pressing for ICS. Since, however, mean pressing/5 min is similar across tests with these compounds and in other tests of opioid-effects [22,25], tentative conclusions can be drawn concerning their relative effects. Win 42,156, Win 44,156, and Win 44,441, as well as morphine, facilitate pressing for lateral hypothalamic ICS and naloxone suppresses pressing. Morphine's minimally effective dose ranges from 0.5 to 2.0 mg/kg and Win 42,156 and Win 44,156's minimally effective doses are less than 0.25 mg/kg in similar tests.

Naloxone reverses the effects of each of the Win compounds as well as morphine and the net effect of a compound plus naloxone is similar to that of naloxone alone. Win 44,441 reduces the effects of the other two Win compounds leaving a net effect similar to an average of each drugs' effect. Win 44,441 clearly antagonized morphine's suppressive effects on pressing for ICS. Taken together, these observations lead to the conclusion that the mode of action of the Win compounds involves specific interaction with opiate receptors.

The three Win compounds have similar molecular structure but different profiles of activity: Win 44,441 has been classed as a pure antagonist, Win 44,156 as a pure agonist, and Win 42,156 as a mixed-agonist-antagonist [18]. Michne *et al.* [18], based on results from tests with Win 44,441 in bioassays (using Guinea-pig ileum and mouse van deferens) and in studies of antagonism of opioid analgesia, concluded that Win 44,441 was one of "the most potent pure antagonists" ([18], p. 205). Win 44,156 shows potent analgesic activity in all analgesic tests. Win 42,156 shows potent analgesia in the tests involving infusions of bradykinin and acetylcholine but not in the tail flick test using rats; a pattern typical of mixed agonist-antagonist drugs. Yet, all three compounds clearly facilitate pressing for ICS, a property common to drugs that will be self-administered [8].

Among the implications of these results is the apparent disassociation between analgesic properties of opioids and their ability to modify pressing for ICS. There is no concordance between a drug's ability to produce analgesia and its ability to facilitate pressing for ICS (these experiments and [9, 14, 21]). If facilitation in pressing for ICS is a sign of a drug's potential to produce positive affect [24], then opioid-analgesia and opioid-positive affect are disassociable. In other words, the subclass of receptor proposed by Martin *et al.* [17] called a mu, or morphine receptor, and related to analgesia and "euphoric subjective effects" ([17], p. 24) may

consist of two types of receptors, one for analgesia and one for positive affect ("euphoria") as indexed by tests of pressing for ICS. Another possibility is that a system of classifying opioid receptors may emerge with concordant data from binding studies, bioassays, and behavioral tests leading to a schema that has one receptor class exclusive to analgesia and one receptor class exclusive to opioid-reinforcement.

ACKNOWLEDGEMENTS

This research was conducted at RPI while the first two authors were supported by Consejo Nacional de Ciencia y Technologia, Mexico, and by funds made available from RPI. We appreciate the advice of Dr. William Michne, Sterling-Winthrop Research Institute, and appreciate the gifts of the WIN-compounds. We acknowledge, with great appreciation, J. Bestle for help with the preparation of the manuscript.

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